Module 3:
Overview of HIV Testing Technologies

Learning Objectives

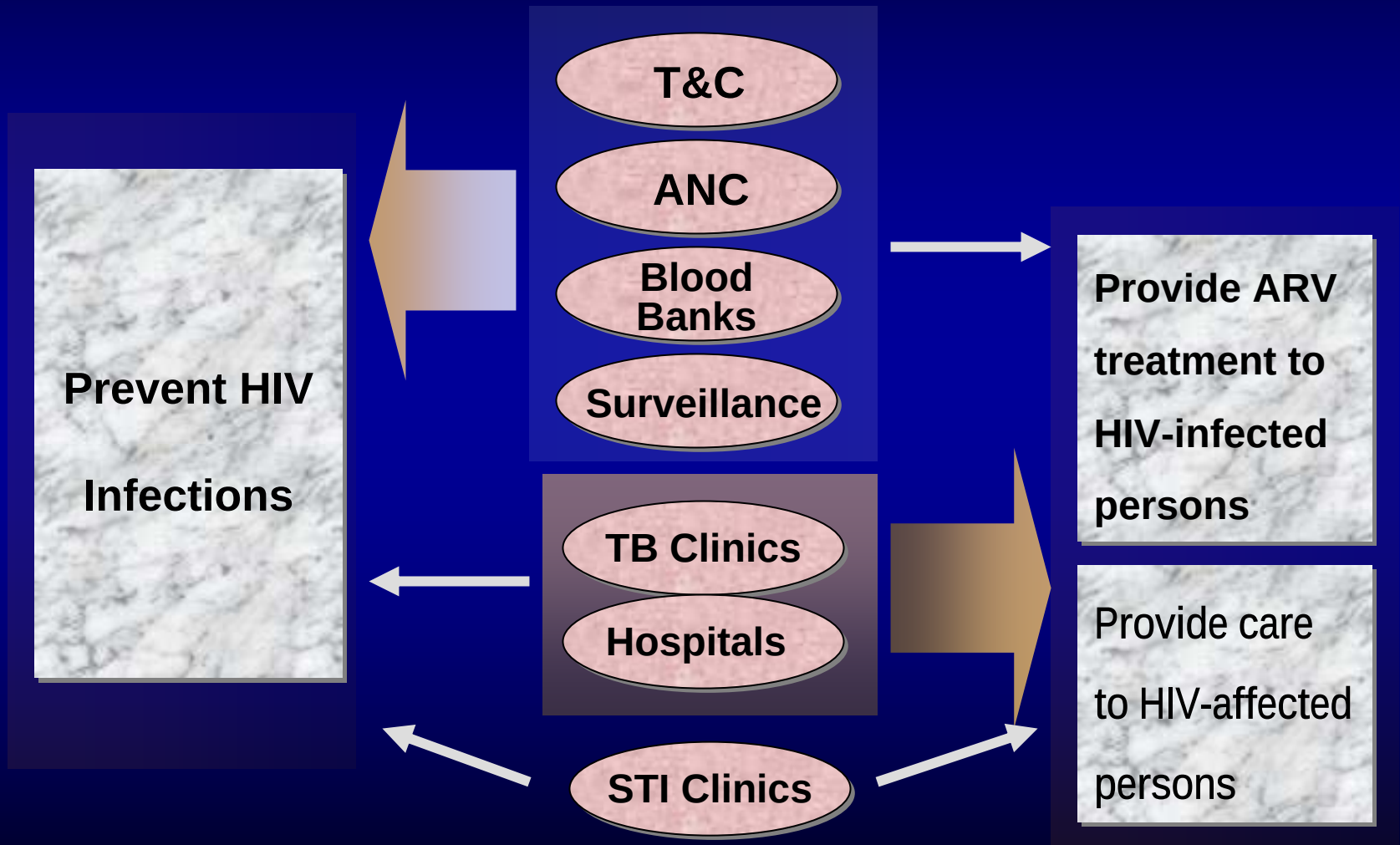
At the end of this module, you will be able to:

- Discuss settings where HIV testing will be part of service delivery during an era of expanded services
- Discuss the spectrum of testing technologies for HIV
- Explain the advantages and disadvantages of HIV rapid tests
- Accurately recognize individual test result as reactive, non-reactive, or invalid

Content Overview

- Expansion of HIV rapid testing
- Spectrum of HIV diagnostic tests
- Challenges with HIV testing
- Spectrum of HIV testing technologies
- Advantages and disadvantages of HIV rapid testing
- Three formats of rapid tests
- Reading individual test results

HIV Testing Occurs in a Variety of Settings



- Lab workers
- Health workers
- Counselors

Expansion of Testing Services

- Integrate HIV laboratory services and diagnostics fully into national health laboratory structures
- Facilitate testing in non-traditional settings
- Consider all our testing options

Lab workers Health workers Counselors

Use of HIV Testing Technologies in the Continuum of Care

Use HIV rapid diagnostic test to identify an HIV infection

Initiate treatment with ARVs minimal diagnostics required

Monitor effectiveness of ARVs with diagnostics (viral load, CD4) and safety with basic laboratory tests

- Lab workers
- Health workers
- Counselors

Spectrum of HIV Tests

- HIV diagnosis (Antibody/Antigen testing)
  - Enzyme Immunoassays (EIAs)
  - Rapid tests
  - Western blot (WB)
- Early diagnosis in infants
  - p24
- Initiation and monitoring of ART
  - CD4
  - Viral Load

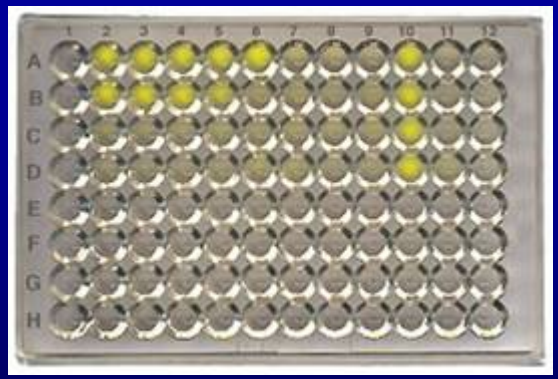
Challenges of HIV Testing

- Early detection of seroconversion
- Early detection in infants born to HIV positive mothers
- Effect of HIV subtypes on test performance
- Impact of other health conditions on test performance
- Product specific equipment
- Technical skill

Enzyme Immunoassays (EIAs)

- Quantitative assay to measure HIV antibodies
  - Most detect antibodies to HIV-1 and HIV-2
  - Antigens coated in microwells
  - HIV Antigen / Antibody reaction is detected by color change
  - Intensity of color reflects amount of antibody present serum
- Some assays can detect both HIV antibody and HIV antigen (close window period)
- Issues:
  - Skilled lab technician
  - Large volume testing
  - Properly maintained equipment required

Enzyme ImmunoAssays (EIAs) – Cont'd



After several incubation and wash steps, a color reaction occurs if HIV antibody is present

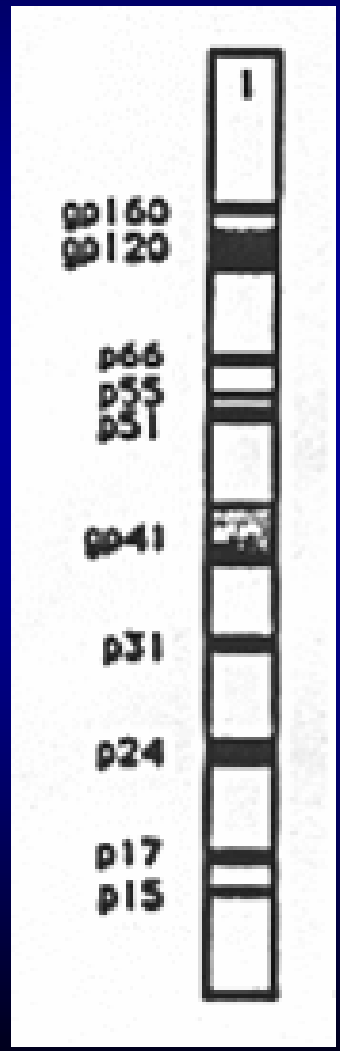
An automated reader gives a measurement of optical density (presence of color) for each well

HIV Rapid Tests

- Qualitative assay to detect HIV antibodies
- Most detect HIV 1 and HIV 2
- As reliable as EIAs
- Issues:
  - Small volumes
  - Validation of use
  - Appropriate training

Western Blot / Line Immunoassays

- Used as supplemental test for confirmation (only difficult cases)
- Detects antibodies to specific HIV antigens on cellulose strip
- Issues:
  - Multiple standards for performance and interpretation
  - Expensive
  - Limited commercial availability



HIV p24 Antigen

- Core protein of the virus
- EIA detects p24 antigen before antibody can be detected
  - Detected 2 to 3 weeks after HIV infection
  - Detected about 6 days before antibody tests become reactive
- Used for:
  - Diagnosis of pediatric HIV-1 infections
  - Blood bank safety (high incidence countries)
- Issues:
  - Level 4 complexity
  - Properly maintained equipment required

CD4 T-Lymphocyte

- CD4 T-lymphocyte counts used for:
  - Determining clinical prognosis
  - Assessing criteria for antiretroviral therapy
  - Monitoring therapy
- Manual and automated methods
- Issues:
  - Requires high level of technical skill for test performance and interpretation
  - Properly maintained equipment

Viral Load

- Quantitative molecular assay measures amount of HIV in blood products
- Used to:
  - Predict disease progression
  - Assist with deciding when to initiate anti-retroviral therapy
  - Monitors response to anti-retrovirals
- Issues:
  - Expensive
  - Labor-intensive
  - Special facilities

Complexity of HIV Tests Varies*

- Level 1: No additional equipment and little or no laboratory experience needed
- Level 2: Reagent preparation or a multi-step process is required; Centrifugation or optimal equipment
- Level 3: Specific skills such as diluting are required
- Level 4: Equipment and trained laboratory technician are required

*WHO Reports

HIV Rapid Tests provides excellent tool for expansion of services

- Lab workers
- Health workers
- Counselors

HIV Rapid Tests: Advantages

- Increases access to prevention (VCT) and interventions (PMTCT)
- Supports increased number of testing sites
- Same-day diagnosis and counseling
- Robust and easy to use
- Test time under 30 minutes
- Most require no refrigeration
- None or one reagent
- Minimal or no equipment required
- Minimum technical skill

HIV Rapid Tests: Disadvantages

- Small numbers for each test run
- Quality Assurance/Quality Control at multiple sites
- Test performance varies by product
- Refrigeration required by some products, e.g., Capillus
- Reader variability in interpretation of results
- Limited end-point stability of test results

Body Fluids Used for HIV Rapid Testing

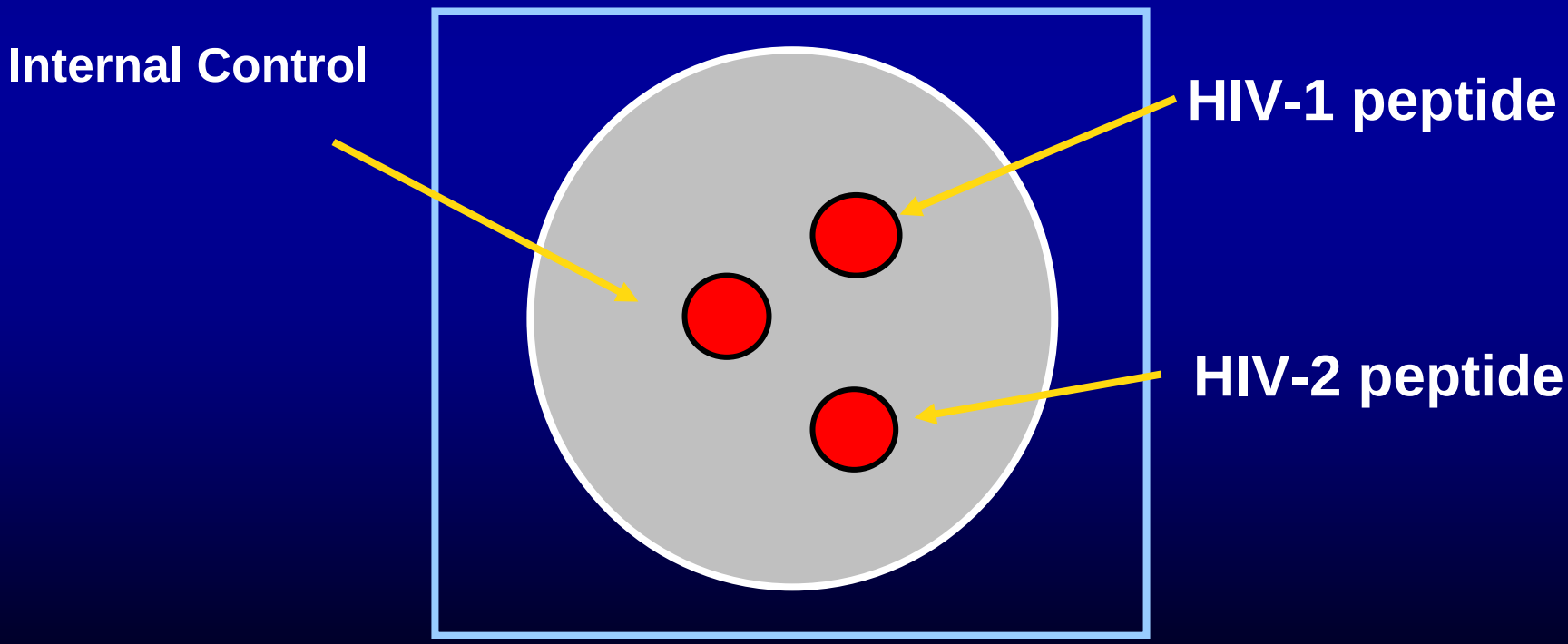
- Serum
- Plasma
- Whole blood
- Oral fluids

Three Formats of HIV Rapid Tests

- Immunoconcentration (flow-through device)
- Immunochromatography (lateral flow)
- Particle agglutination

How Immunoconcentration Works

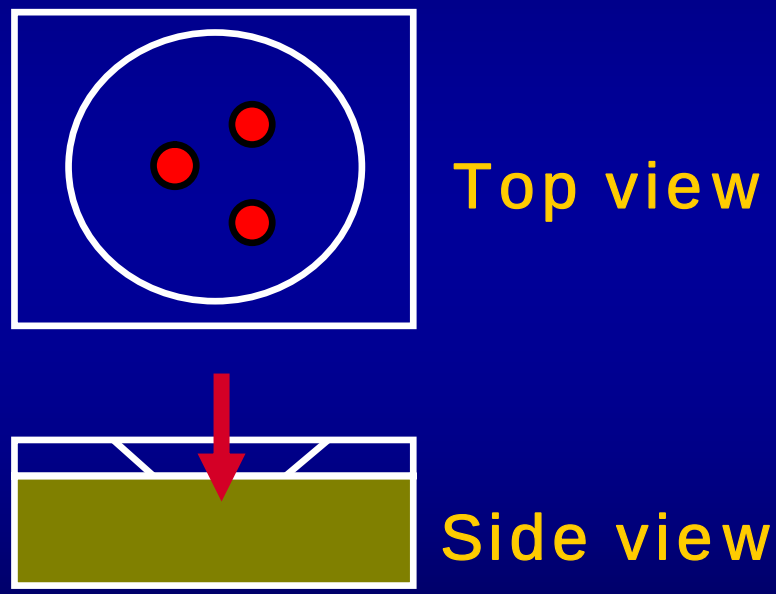
HIV antibody links to bound HIV peptide antigens forming the color spot



Tests Based on Immunoconcentration

Flow-Through Devices:

- Multi-Spot
- Genie II



Reading Results: Genie II

Non-reactive

Reactive

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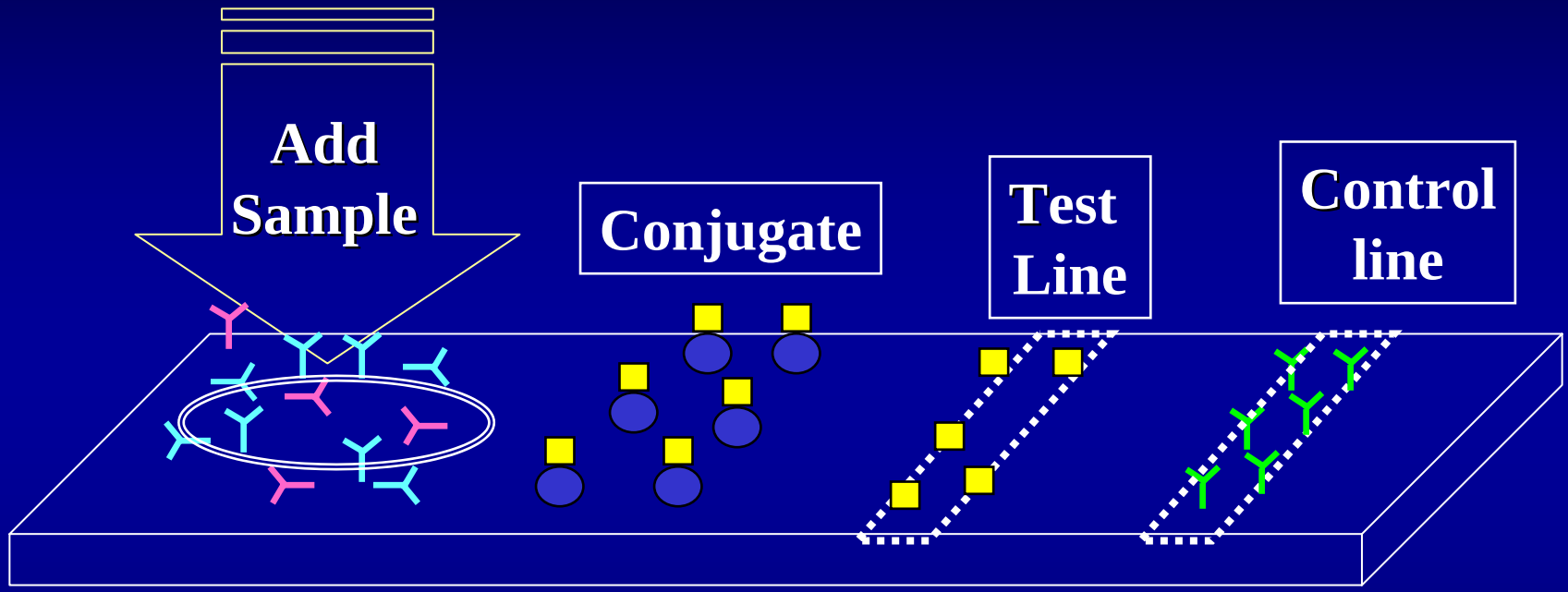
How Immunochromatography Works



IgG Antibodies
HIV antibodies

Colloidal gold conjugated to HIV antigen

HIV antigen

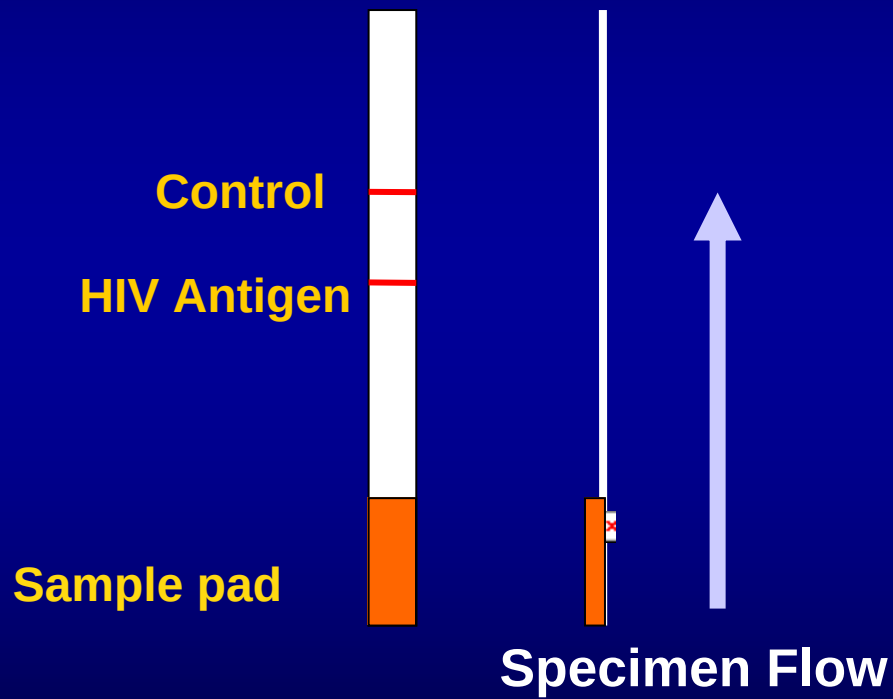
Anti-IgG/gold antibodies

Lab workers Health workers

Tests Based on Immunochromatography

Lateral Flow Devices

- Determine
- Hema-Strip
- OraQuick
- Unigold



Reading Results: Determine

Non-Reactive

Reactive

Reading Results: OraQuick

Non-Reactive

Reactive

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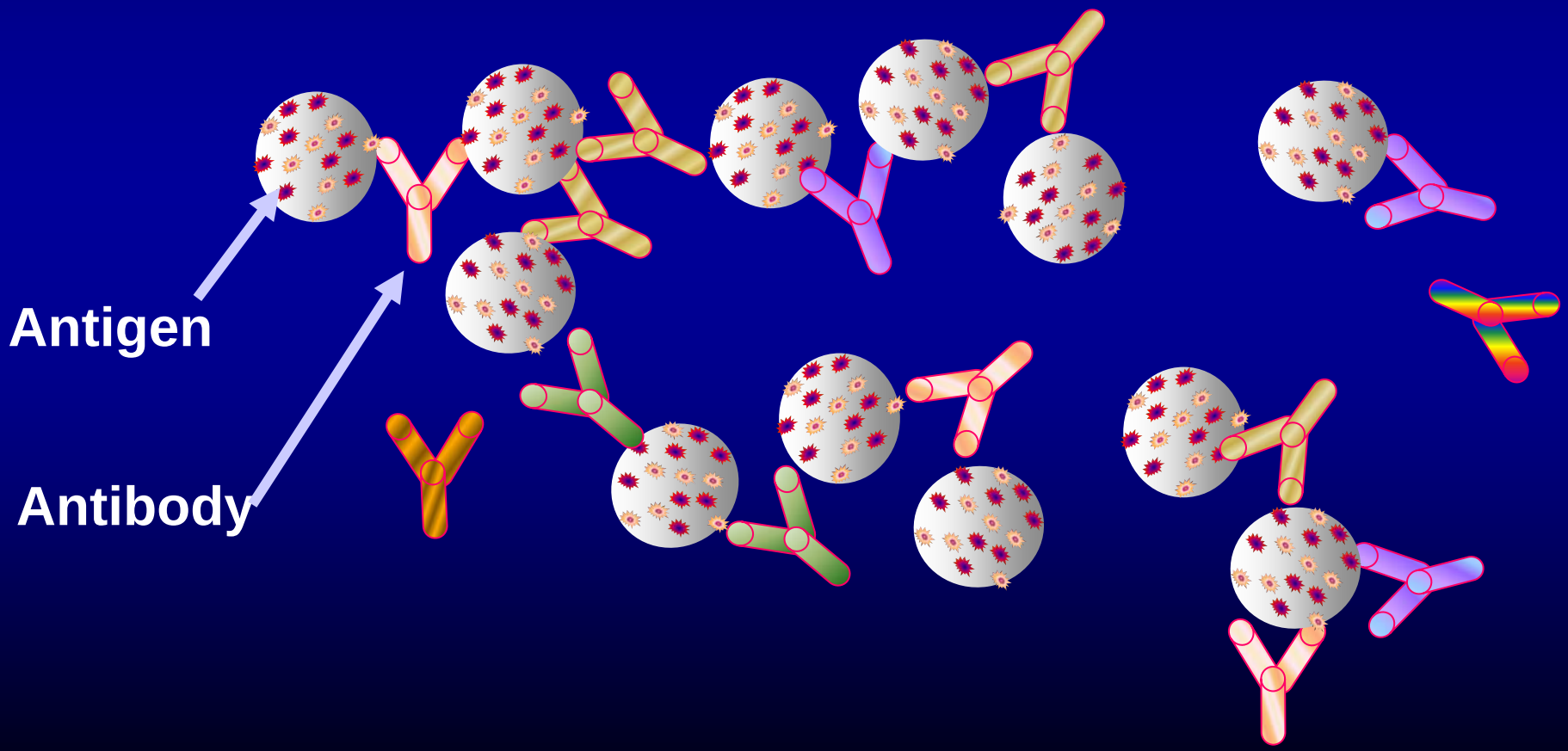
How Particle Agglutination Works

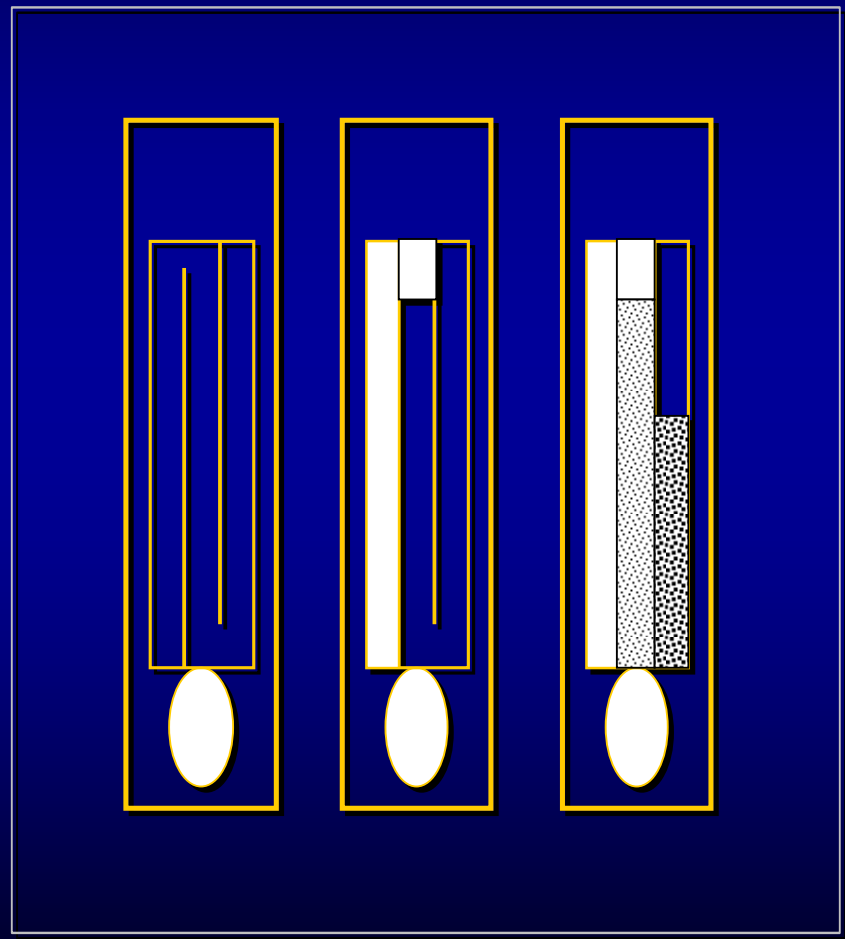
Anti-HIV antibodies bind to the antigen-coated latex particles.



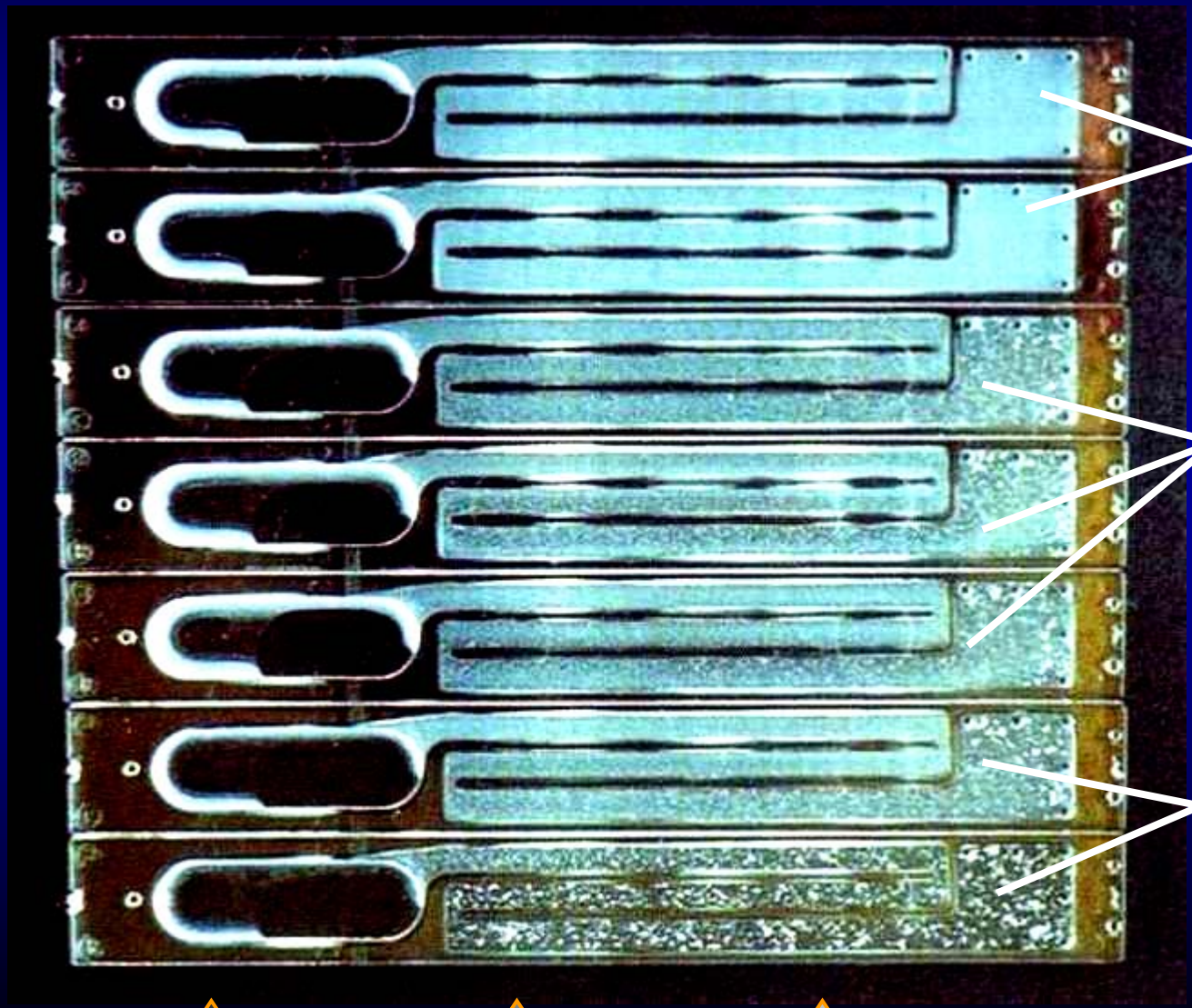
Tests Based On Agglutination

Agglutination devices:

- Capillus
- Serodia



Reading Results: Capillus



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There Are Only Three Possible Outcomes for Single HIV Antibody Tests

Reactive or "Positive"

- Test band
- Control band

Non-reactive or "Negative"

- Control band only

Invalid

- No control band present
- Test has failed. Repeat with new device.

Exercise: Interpreting Individual HIV Rapid Test Results

- *Refer to Participant Manual*
- *Work alone to determine individual test results*
- *3 Minutes*

Key Messages

- HIV rapid tests can be as reliable as EIA
- All tests require attention to training, supervision, and monitoring at points of service.
- As testing is expanding and decentralized, training, supervision, and monitoring must follow accordingly and become all the more important.

Summary

- Where is HIV rapid testing likely to occur during an era of expansion of services?
- What is the intended use for: EIAs, Western Blot, p24 Antigen, CD4, Viral Load
- What are rapid tests?
- Why use rapid tests?
- How do you read a result – reactive, non-reactive or invalid?